

PATENT APPLICATION
Mo6411
LeA 34,261

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICATION OF)
OTTO SCHALLNER ET AL)
SERIAL NUMBER: TO BE ASSIGNED)
FILED: HEREWITH)
TITLE: METHOD OF FINDING)
PROTOPORPHYRINOGEN)
OXIDASE INHIBITORS)

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents

Washington, D.C. 20231

Dear Sir:

Prior to calculation of the filing fee and examination of the present application,
please amend the application as follows:

"Express Mail" mailing label number ET146894886US

Date of Deposit July 2, 2001

I hereby certify that this paper or fee is being deposited with the United States
Postal Service "Express Mail Post Office to Addressee" service under 37 CFR
1.10 on the date indicated above and is addressed to the Assistant Commissioner
of Patents and Trademarks, Washington, D.C. 20231

Dorothy P. Colangelo

(Name of person mailing paper or fee)

Dorothy P. Colangelo

(Signature of person mailing paper or fee)

In the Specification:

Please amend the specification as follows:

On page 1, after the title, please insert:

--FIELD OF THE INVENTION--

On page 1, after line 7, please insert:

--BACKGROUND OF THE INVENTION--

On page 2, after line 21, please insert:

--SUMMARY OF THE INVENTION

Methods for finding substances which interact with the enzyme protoporphyrinogen oxidase (PPO) comprise the steps of preparing mixtures which comprise PPO and a substance which fluoresces, and irradiating the mixtures with plane-polarized light.

DETAILED DESCRIPTION--

In the Claims:

Please cancel Claim 24.

Please amend Claims 1-23 and 25 as follows

1. (Amended) A method for finding substances which interact with the enzyme protoporphyrinogen oxidase (PPO) comprising the steps of:
 - a) preparing mixtures which comprise, in various concentrations, (i) PPO, (ii) a substance which is capable of interacting with PPO and which fluoresces when exposed to suitable irradiation, and (iii) a substance to be tested, or a mixture of substances to be tested,
 - b) irradiating the mixtures with plane-polarized light of a suitable wavelength which excites the fluorescent substance to emit light, and
 - c) measuring the fluorescence polarization values or the anisotropy values of the light emitted,where a decrease in the fluorescence polarization value with an increasing concentration of the substance to be tested or of the mixture of substances to be tested indicates an interaction of one or more substances to be tested with PPO.

! -

2. (Amended) A method for assaying whether a substance interacts with the enzyme protoporphyrinogen oxidase (PPO) comprising the steps of:

- preparing mixtures which comprise, in various concentrations, (i) PPO and (ii) a substance which fluoresces when exposed to suitable irradiation,
- irradiating the mixtures with plane-polarized light of a suitable wavelength which excites the fluorescent substance to emit light,
- measuring the fluorescence polarization values or the anisotropy values of the light emitted,
where an increase in the fluorescence polarization value with a decreasing concentration of the fluorescent substance indicates an interaction with PPO.

3. (Amended) A method according to Claim 1, wherein the PPO is a plant PPO.

4. (Amended) A method according to Claim 1, wherein a PPO-containing plant cell extract is employed.

5. (Amended) A method according to Claim 1, wherein biochemically purified PPO from plant cell extracts is employed.

6. (Amended) A method according to Claim 1, wherein recombinantly produced PPO is employed.

7. (Amended) A method according to Claim 1, wherein the fluorescent substance is a substance labelled with a fluorescent dye.

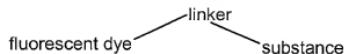
8. (Amended) A method according to Claim 7, wherein the fluorescent dye is fluorescein or a fluorescein derivative.

9. (Amended) A method according to Claim 1, wherein the substance which is capable of interacting with PPO is a PPO ligand, a natural PPO substrate, a

(1) 100% 200% 300% 400%
500% 600% 700% 800%
900% 1000% 1100% 1200%

natural product of the PPO enzyme reaction or a herbicidally active PPO inhibitor.

10. (Amended) A method according to Claim 1, wherein the fluorescent substance has the following structure:



where

"linker"

represents a hydrocarbon chain which is in each case straight-chain or branched, in each case saturated or unsaturated, in each case optionally substituted, in each case linked at one end to the substance and at the other end to the fluorescent dye, it being possible for this hydrocarbon chain to contain in each case at the beginning or at the end or within the chain one or more of the following hetero components:

Q^1 , CQ^2 , CQ^2-Q^1 , Q^1-CQ^2 , $Q^1-CQ^2-Q^1$, SO , SO_2

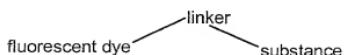
where in each case Q^1 and Q^2 represent O, S or NH,

or represents a carbocyclic or heterocyclic group which is in each case saturated or unsaturated, in each case optionally substituted and linked at one end to the substance and at the other end to the fluorescent dye, and

"substance"

represents a substance which is capable of interacting with PPO.

11. (Amended) A substance labelled with a fluorescent dye, having the structure:



where

"linker"

represents a hydrocarbon chain which is in each case straight-chain or branched, in each case saturated or unsaturated, in each case optionally substituted, in each case linked at one end to the substance and at the other end to the fluorescent dye, it being possible for this hydrocarbon chain to contain in each case at the beginning or at the end or within the chain one or more of the following hetero components:

Q^1 , CQ^2 , CQ^2-Q^1 , Q^1-CQ^2 , $Q^1-CQ^2-Q^1$, SO, SO_2

where in each case Q^1 and Q^2 represent O, S or NH,

or represents a carbocyclic or heterocyclic group which is in each case saturated or unsaturated, in each case optionally substituted and linked at one end to the substance and at the other end to the fluorescent dye, and

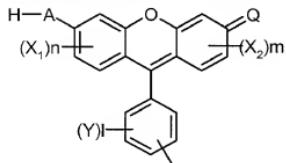
"substance"

represents a substance which is capable of interacting with PPO.

12. (Amended) A substance according to Claim 11, wherein

"fluorescent dye"

represents a dye group having the formula



where

A represents O or NH,

Q represents O, S or NH,

X¹ represents hydrogen, halogen, nitro, hydroxyl, carboxyl (COOH), sulpho (SO₃H) or alkyl,

X² represents hydrogen, halogen, nitro, hydroxyl, carboxyl (COOH), sulpho (SO₃H) or alkyl,

Y represents halogen, alkyl, carboxyl (COOH) or sulpho (SO₃H),

l represents the indices 0 to 4, and

m, n represents the indices 0 to 3.

13. (Amended) A substance according to Claim 12, wherein

A represents O.

14. (Amended) A substance according to Claim 12, wherein

Q represents O.

15. (Amended) A substance according to Claim 12, wherein

X¹ represents hydrogen, fluorine, chlorine, bromine or alkyl having 1 to 4 carbon atoms, and

X² represents hydrogen, fluorine, chlorine, bromine or alkyl having 1 to 4 carbon atoms.

16. (Amended) A substance according to Claim 12, wherein

X¹ represents hydrogen, fluorine, chlorine, methyl or ethyl, and

X² represents hydrogen, fluorine, chlorine, methyl or ethyl.

17. (Amended) A substance according to Claim 11, wherein the hydrocarbon chain of the linker has up to 10 carbon atoms.

18. (Amended) A substance according to Claim 11, wherein the carbocyclic or heterocyclic group of the linker has up to 10 carbon atoms and up to 5 nitrogen atoms.

19. (Amended) A substance according to Claim 11, wherein the substance has the structure:



where

A¹ represents a single bond, O or S,

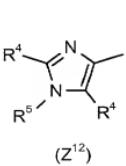
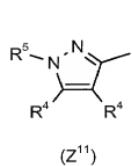
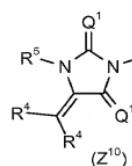
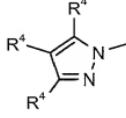
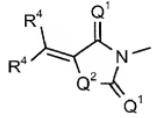
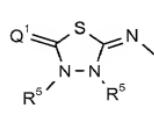
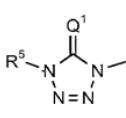
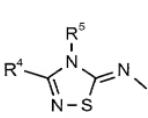
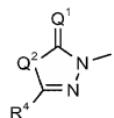
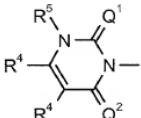
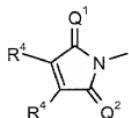
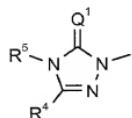
Z¹ represents a carbocyclic or heterocyclic group having in each case up to 10 carbon atoms and, if appropriate, up to 5 nitrogen atoms and, if appropriate, 1 or 2 oxygen or sulphur atoms, and

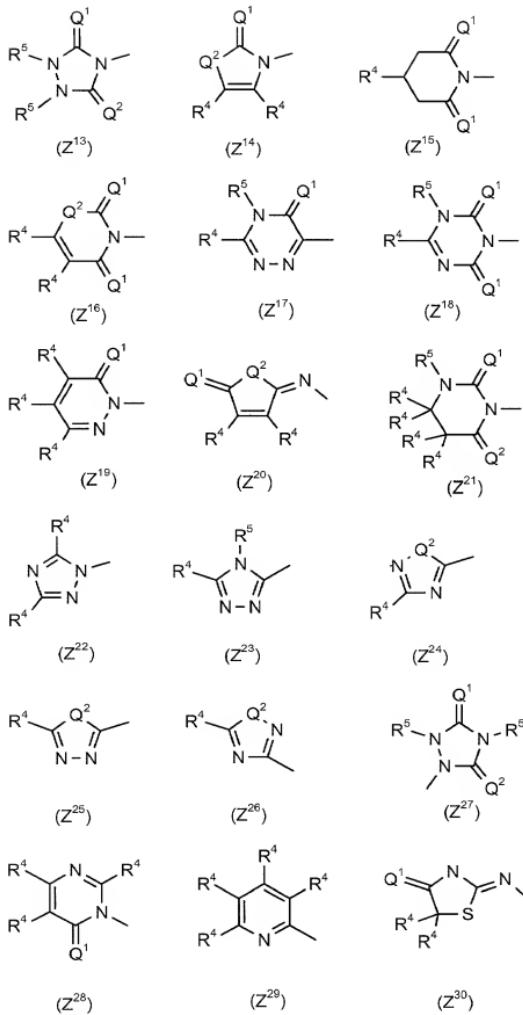
Z² represents a carbocyclic or heterocyclic group having in each case up to 10 carbon atoms and, if appropriate, up to 5 nitrogen atoms and, if appropriate, 1 or 2 oxygen or sulphur atoms.

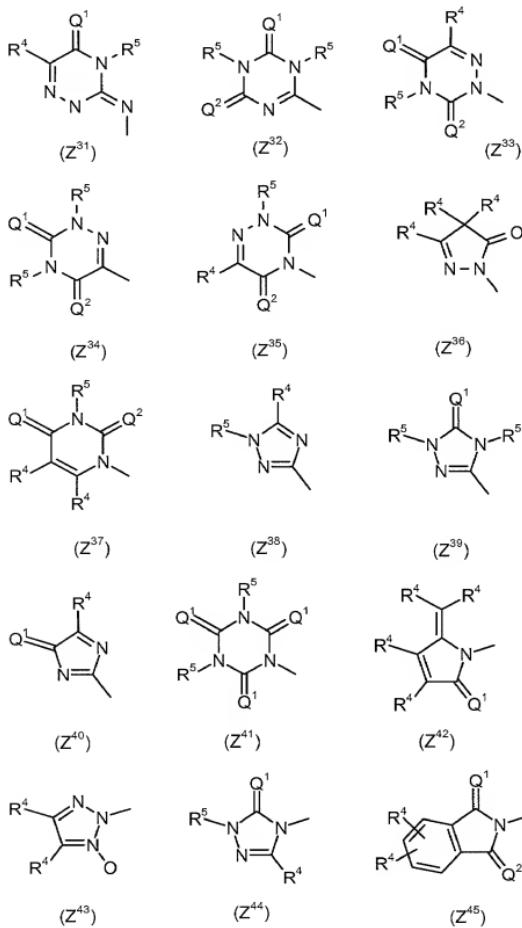
20. (Amended) A substance according to Claim 19, wherein

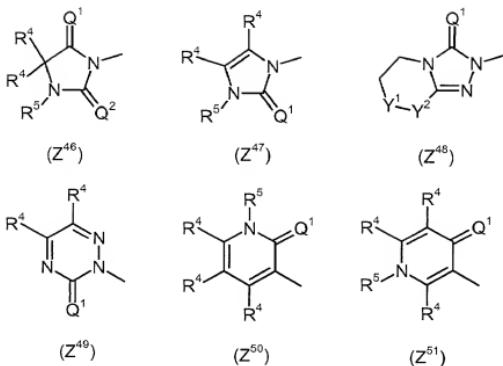
A¹ represents a single bond,

Z¹ represents one of the following groups









where

Q^1 represents O or S,

Q^2 represents O or S,

R^4 represents hydrogen, amino, nitro, cyano, carboxyl, carbamoyl, thiocarbamoyl, halogen, alkyl which has 1 to 6 carbon atoms and which is optionally substituted by cyano, halogen or $C_1\text{-}C_4$ -alkoxy, or represents alkenyl or alkynyl, each of which has 2 to 6 carbon atoms and each of which is optionally substituted by halogen or represents alkoxy or alkoxy carbonyl, each of which has 1 to 6 carbon atoms in the alkyl groups and each of which is optionally substituted by cyano, halogen or $C_1\text{-}C_4$ -alkoxy, or represents alkenyloxy or alkyloxy, each of which has 3 to 6 carbon atoms and each of which is optionally substituted by halogen, or represents alkylthio which has 1 to 6 carbon atoms which is optionally substituted by cyano, halogen or $C_1\text{-}C_4$ -alkoxy, or represents alkenylthio or alkynylthio, each of which has 3 to 6 carbon atoms and each of which is optionally substituted by halogen, or

represents alkylamino or dialkylamino, each of which has 1 to 6 carbon atoms in the alkyl groups, or represents cycloalkyl or cycloalkylalkyl, each of which has 3 to 6 carbon atoms in the cycloalkyl groups and, if appropriate, 1 to 4 carbon atoms in the alkyl moiety and each of which is optionally substituted by cyano, halogen or C₁-C₄-alkyl,

R⁵ represents hydrogen, hydroxyl, amino, cyano, or represents alkyl, alkoxy, alkoxycarbonyl or alkylamino, each of which has up to 6 carbon atoms and each of which is optionally substituted by cyano, halogen or C₁-C₄-alkoxy, or represents alkenyl or alkinyl, each of which has up to 6 carbon atoms and each of which is optionally substituted by halogen, or represents cycloalkyl or cycloalkylalkyl, each of which has 3 to 6 carbon atoms in the cycloalkyl groups and, if appropriate 1 to 4 carbon atoms in the alkyl moiety and each of which is optionally substituted by cyano, halogen or C₁-C₄-alkyl, or represents phenyl or phenyl-C₁-C₄-alkyl each of which is optionally substituted by nitro, cyano, halogen, C₁-C₄-alkyl, C₁-C₄-halogenoalkyl, C₁-C₄-alkoxy or C₁-C₄-halogenoalkoxy,

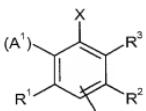
Y¹ represents O, S, SO, SO₂, NH, N(alkyl) or methylene and

Y² represents a single bond or O, S, SO, SO₂, NH or N(alkyl),

Y¹ and Y² being different in each individual case,

and

Z² represents the following group



where

- R¹ is hydrogen, nitro, cyano or halogen,
- R² is nitro, hydroxyl, cyano, carbamoyl, thiocarbamoyl, or represents alkyl or alkoxy, each of which has 1 to 4 carbon atoms and each of which is optionally substituted by halogen, and
- R³ represents nitro, hydroxyl, mercapto, amino, hydroxyamino, cyano, carboxyl, carbamoyl, thiocarbamoyl, halogen, or represents alkyl, alkylcarbonyl, alkoxy, alkoxy carbonyl, alkylthio, alkylsulphinyl, alkylsulphonyl or alkylamino, each of which has 1 to 6 carbon atoms in the alkyl groups and each of which is optionally substituted by cyano, carboxyl, carbamoyl, halogen, C₁-C₄-alkoxy or C₁-C₄-alkoxy-carbonyl, or represents alkylsulphonylamino, N,N-bis-alkylsulphonyl-amino, N-alkylcarbonyl-N-alkylsulphonyl-amino, each of which has 1 to 4 carbon atoms in the alkyl groups and each of which is optionally substituted by halogen, or represents alkenyl, alkenyloxy, alkenylthio, alkenylamino, alkinyl, alkinyloxy, alkinylthio, alkinylamino, each of which has up to 6 carbon atoms in the alkenyl or alkinyl groups and each of which is optionally substituted by cyano, carboxyl, carbamoyl, halogen or C₁-C₄-alkoxy-carbonyl, or represents cycloalkyl, cycloalkyloxy, cycloalkylthio, cycloalkylamino, cycloalkylsulphonylamino, cycloalkylalkyl, cycloalkylalkoxy, cycloalkylalkylthio or cycloalkylalkylamino, each of which has 3 to 6 carbon atoms in the cycloalkyl groups and, if appropriate, 1 to 4 carbon atoms in the alkyl moieties and each of which is optionally substituted by cyano, carboxyl, carbamoyl, halogen

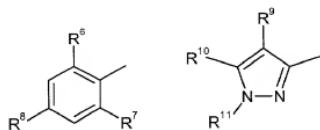
or C₁-C₄-alkyl, or represents aryl, aryloxy, arylthio, arylamino, arylalkyl, arylalkoxy, arylalkylthio, arylalkylamino, N-arylcarbonyl-N-alkylsulphonyl-amino, each of which has 6 or 10 carbon atoms in the aryl groups and, if appropriate, 1 to 4 carbon atoms in the alkyl moiety and each of which is optionally substituted by nitro, cyano, carboxyl, carbamoyl, halogen, C₁-C₄-alkyl, C₁-C₄-halogenoalkyl, C₁-C₄-alkoxy, C₁-C₄-halogenoalkoxy or C₁-C₄-alkoxy-carbonyl, and

X represents hydrogen or halogen.

21. (Amended) A substance according to Claim 19, wherein

A¹ represents O,

Z¹ represents one of the following groups



where

R⁶ represents cyano or halogen,

R⁷ represents hydrogen or halogen,

R⁸ represents cyano, halogen, or represents halogenoalkyl or halogenoalkoxy, each of which has 1 to 4 carbon atoms,

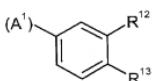
R⁹ represents hydrogen or halogen,

R¹⁰ represents halogenoalkyl or halogenoalkoxy, each of which has 1 to 4 carbon atoms, and

R¹¹ represents alkyl having 1 to 4 carbon atoms,

and

Z² represents the following group

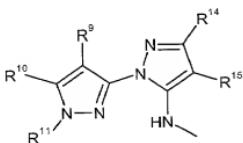


wherein

R¹² represents carboxyl, or represents alkoxy or alkoxycarbonyl, each of which has up to 4 carbon atoms and each of which is optionally substituted by cyano, halogen or C₁-C₄-alkoxy, and

R¹³ represents cyano, carbamoyl, thiocarbamoyl, halogen or represents halogenoalkyl or halogenoalkoxy, each of which has 1 to 4 carbon atoms.

22. (Amended) A substance according to Claim 19, wherein the substance has the following general formula:



wherein

R⁹ represents hydrogen or halogen,

R¹⁰ represents halogenoalkyl or halogenoalkoxy, each of which has 1 to 4 carbon atoms, and

R¹¹ represents alkyl having 1 to 4 carbon atoms,

R¹⁴ represents hydrogen, halogen or alkyl having 1 to 4 carbon atoms, and

R¹⁵ represents nitro, cyano, carbamoyl, thiocarbamoyl, or represents alkyl or alkoxy, each of which has 1 to 4 carbon atoms and each of which is optionally substituted by cyano, halogen or C₁-C₄-alkoxy.

23. (Amended) An assay system comprising

- a) containers with mixtures as defined in Claim 1,
- b) a device for irradiating the mixtures of plane-polarized light of a wavelength which excites the fluorescent substance to emit light, and
- c) a device for measuring the fluorescence polarization values or the anisotropy values of the light emitted.

25. (Amended) A substance which interact with the enzyme protoporphyrinogen oxidase and which have been identified by the method according to Claim 1.

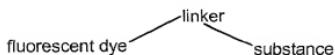
Please add the following claims:

--26. A method according to Claim 2, wherein the PPO is a plant PPO.

27. A method according to Claim 2, wherein a PPO-containing plant cell extract is employed.

TOKYO 20060

- 28. A method according to Claim 2, wherein biochemically purified PPO from plant cell extracts is employed.
- 29. A method according to Claim 2, wherein recombinantly produced PPO is employed.
- 30. A method according to Claim 2, wherein the fluorescent substance is a substance labelled with a fluorescent dye.
- 31. A method according to Claim 2, wherein the fluorescent substance has the following structure:



wherein "linker" represents a hydrocarbon chain which is in each case straight-chain or branched, in each case saturated or unsaturated, in each case optionally substituted, in each case linked at one end to the substance and at the other end to the fluorescent dye, it being possible for this hydrocarbon chain to contain in each case at the beginning or at the end or within the chain one or more of the following hetero components:



wherein Q^1 and Q^2 represent O, S or NH, or represents a carbocyclic or heterocyclic group which is in each case saturated or unsaturated, in each case optionally substituted and linked at one end to the substance and at the other end to the fluorescent dye, and

"substance" represents a substance which is capable of interacting with PPO.

- 32. A substance which interact with the enzyme protoporphyrinogen oxidase and which have been identified by the method according to Claim 2.
- 33. A substance according to Claim 12, wherein

A represents O.
Q represents O.
 X^1 represents hydrogen, fluorine, chlorine, bromine or alkyl having 1 to 4 carbon atoms, and
 X^2 represents hydrogen, fluorine, chlorine, bromine or alkyl having 1 to 4 carbon atoms.

34. A substance according to Claim 11, wherein the carbocyclic or heterocyclic group of the linker has up to 10 carbon atoms and 1 or 2 oxygen or sulphur atoms.

35. A substance according to Claim 11, wherein the carbocyclic or heterocyclic group of the linker has up to 10 carbon atoms, up to 5 nitrogen atoms, and 1 or 2 oxygen or sulphur atoms.

36. An assay system comprising
a) containers with mixtures as defined in Claim 2,
b) a device for irradiating the mixtures of plane-polarized light of a wavelength which excites the fluorescent substance to emit light, and
c) a device for measuring the fluorescence polarization values or the anisotropy values of the light emitted.--

REMARKS

By present amendment the claims have been amended to present the claims in accordance with customary U.S. practice, care having been exercised to avoid any introduction of new matter.

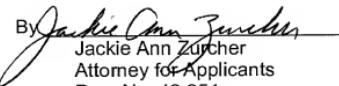
Claim 24 has been canceled, and Claims 1-23 and 25 have been amended as to form. Claims 26-36 have been added. Support for Claims 26-32 can be found in original Claims 3-7, 10, and 25, respectively. Support for Claim 33 can be found in original Claims 13-15, while support for Claims 34-35 can be found in original Claim 18, and support for Claim 36 can be found in original Claim 23. The amendments to the claims remove multiple dependencies and place the claims in United States form.

The specification has been amended to include section headings in accordance with customary U.S. practice, and to include a Summary of the Invention. Support for the amendment to the specification can be found in original Claim 1.

The amendments to the claims and the specification do not involve any introduction of new matter, whereby entry is believed to be in order and is respectively requested.

Attached is a marked-up version of the changes made to the specification and claims by the current amendment. The attached page is captioned "Version With Markings To Show Changes Made".

Respectfully submitted,

By 
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/jme/JAZ0097

VERSION WITH MARKINGS TO SHOW CHANGES MADE

In the Specification:

On page 1, after the title, was inserted:

--FIELD OF THE INVENTION--

On page 1, after line 7, was inserted:

--BACKGROUND OF THE INVENTION--

On page 2, after line 21, was inserted:

--SUMMARY OF THE INVENTION

Methods for finding substances which interact with the enzyme protoporphyrinogen oxidase (PPO) comprise the steps of preparing mixtures which comprise PPO and a substance which fluoresces, and irradiating the mixtures with plane-polarized light.

DETAILED DESCRIPTION--

In the Claims:

Claim 24 has been canceled.

Claims 1-23 and 25 have been amended as follows:

1. (Amended) A m[M]ethod for finding substances which interact with the enzyme protoporphyrinogen oxidase (PPO) comprising the [following steps of:
 - a) preparing mixtures which comprise, in various concentrations, (i) PPO, (ii) a substance which is capable of interacting with PPO and which fluoresces when exposed to suitable irradiation, and (iii) a substance to be tested, or a mixture of substances to be tested,
 - b) irradiating the mixtures with plane-polarized light of a suitable wavelength which excites the fluorescent substance to emit light, and
 - c) measuring the fluorescence polarization values or the anisotropy values of the light emitted,where a decrease in the fluorescence polarization value with an increasing concentration of the substance to be tested or of the mixture of substances to be tested indicates an interaction of one or more substances to be tested with

PPO.

2. (Amended) A m[M]ethod for assaying whether a substance interacts with the enzyme protoporphyrinogen oxidase (PPO) comprising the [following] steps of :

- preparing mixtures which comprise, in various concentrations, (i) PPO and (ii) a substance which fluoresces when exposed to suitable irradiation,
- irradiating the mixtures with plane-polarized light of a suitable wavelength which excites the fluorescent substance to emit light,
- measuring the fluorescence polarization values or the anisotropy values of the light emitted,

where an increase in the fluorescence polarization value with a decreasing concentration of the fluorescent substance indicates an interaction with PPO.

3. (Amended) A m[M]ethod according to Claim 1, wherein [or 2, characterized in that] the PPO is a plant PPO.

4. (Amended) A m[M]ethod according to Claim 1, wherein [one of Claims 1 to 3, characterized in that] a PPO-containing plant cell extract is employed.

5. (Amended) A m[M]ethod according to Claim 1, wherein [one of Claims 1 to 3, characterized in that] biochemically purified PPO from plant cell extracts is employed.

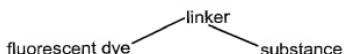
6. (Amended) A m[M]ethod according to Claim 1, wherein [one of Claims 1 to 3, characterized in that] recombinantly produced PPO is employed.

7. (Amended) A m[M]ethod according to Claim 1, wherein [one of Claims 1 to 6, characterized in that] the fluorescent substance is a substance labelled with a fluorescent dye.

8. (Amended) A m[M]ethod according to Claim 7, [characterized in that] wherein the fluorescent dye is fluorescein or a fluorescein derivative.

9. (Amended) A m[M]ethod according to Claim 1, wherein [one of Claims 1 to 8, characterized in that] the substance which is capable of interacting with PPO is a PPO ligand, a natural PPO substrate, a natural product of the PPO enzyme reaction or a herbicidally active PPO inhibitor.

10. (Amended) A m[M]ethod according to Claim 1, wherein [one of Claims 1 to 9, characterized in that] the fluorescent substance has the following structure:



where

"linker"

represents a hydrocarbon chain which is in each case straight-chain or branched, in each case saturated or unsaturated, in each case optionally substituted, in each case linked at one end to the substance and at the other end to the fluorescent dye, it being possible for this hydrocarbon chain to contain in each case at the beginning or at the end or within the chain one or more of the following hetero components:



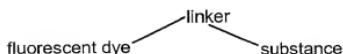
where in each case Q^1 and Q^2 represent O, S or NH,

or represents a carbocyclic or heterocyclic group which is in each case saturated or unsaturated, in each case optionally substituted and linked at one end to the substance and at the other end to the fluorescent dye, and

"substance"

represents a substance which is capable of interacting with PPO.

11. (Amended) A s[S]ubstance labelled with a fluorescent dye, [characterized by the following] having the structure:



where

"linker"

represents a hydrocarbon chain which is in each case straight-chain or branched, in each case saturated or unsaturated, in each case optionally substituted, in each case linked at one end to the substance and at the other end to the fluorescent dye, it being possible for this hydrocarbon chain to contain in each case at the beginning or at the end or within the chain one or more of the following hetero components:

Q^1 , CQ^2 , CQ^2-Q^1 , Q^1-CQ^2 , $Q^1-CQ^2-Q^1$, SO , SO_2

where in each case Q^1 and Q^2 represent O, S or NH,

or represents a carbocyclic or heterocyclic group which is in each case saturated or unsaturated, in each case optionally substituted and linked at one end to the substance and at the other end to the fluorescent dye, and

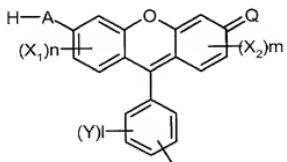
"substance"

represents a substance which is capable of interacting with PPO.

12. (Amended) A s[S]ubstance according to Claim 11, [characterized in that] wherein

"fluorescent dye"

represents a dye group [which is characterized by] having the formula
[hereinbelow]



where

A represents O or NH,

Q represents O, S or NH,

X¹ represents hydrogen, halogen, nitro, hydroxyl, carboxyl (COOH), sulpho (SO₃H) or alkyl,

X² represents hydrogen, halogen, nitro, hydroxyl, carboxyl (COOH), sulpho (SO₃H) or alkyl,

Y represents halogen, alkyl, carboxyl (COOH) or sulpho (SO₃H),

I represents the indices 0 to 4, and

m, n represents the indices 0 to 3.

13. (Amended) A s[S]ubstance according to Claim 12, [characterized in that]

wherein

A represents O.

14. (Amended) A substance according to Claim 12, wherein [or 13, characterized in that]

Q represents O.

15. (Amended) A substance according to Claim 12, wherein [one of Claims 12 to 14, characterized in that]

X¹ represents hydrogen, fluorine, chlorine, bromine or alkyl having 1 to 4 carbon atoms, and

X² represents hydrogen, fluorine, chlorine, bromine or alkyl having 1 to 4 carbon atoms.

16. (Amended) A substance according to Claim 12, wherein [one of Claims 12 to 15, characterized in that]

X¹ represents hydrogen, fluorine, chlorine, methyl or ethyl, and

X² represents hydrogen, fluorine, chlorine, methyl or ethyl.

17. (Amended) A substance according to Claim 11, wherein [one of Claims 11 to 16, characterized in that] the hydrocarbon chain of the linker has up to 10 carbon atoms.

18. (Amended) A substance according to Claim 11, wherein [one of Claims 11 to 17, characterized in that] the carbocyclic or heterocyclic group of the linker has up to 10 carbon atoms and[, if appropriate,] up to 5 nitrogen atoms [and, if appropriate, 1 or 2 oxygen or sulphur atoms].

19. (Amended) A substance according to Claim 11, wherein [one of Claims 11 to 18, characterized in that] the substance has the [following] structure:



where

A¹ represents a single bond, O or S,

Z¹ represents a carbocyclic or heterocyclic group having in each case up to 10 carbon atoms and, if appropriate, up to 5 nitrogen atoms and, if appropriate, 1 or 2 oxygen or sulphur atoms, and

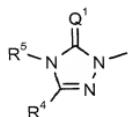
Z² represents a carbocyclic or heterocyclic group having in each case up to 10 carbon atoms and, if appropriate, up to 5 nitrogen atoms and, if appropriate, 1 or 2 oxygen or sulphur atoms.

20. (Amended) A s[S]ubstance according to Claim 19, [characterized in that]

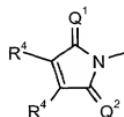
wherein

A¹ represents a single bond,

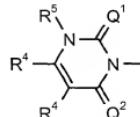
Z¹ represents one of the following groups



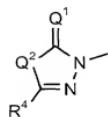
(Z¹)



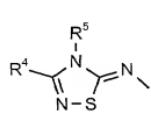
(Z²)



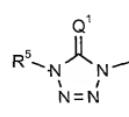
(Z³)



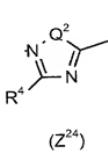
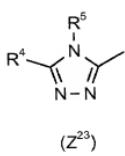
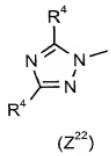
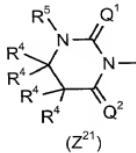
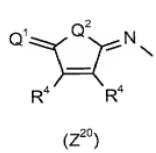
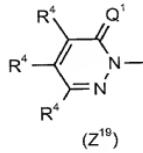
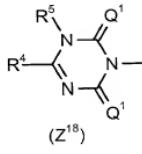
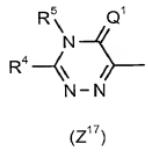
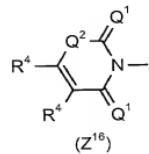
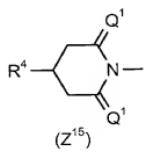
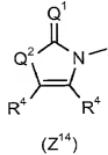
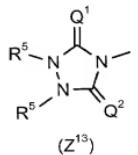
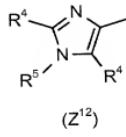
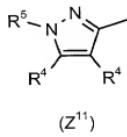
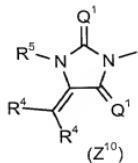
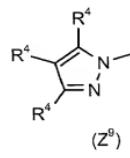
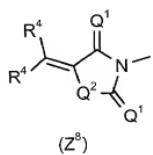
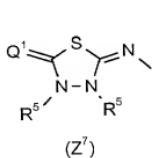
(Z⁴)

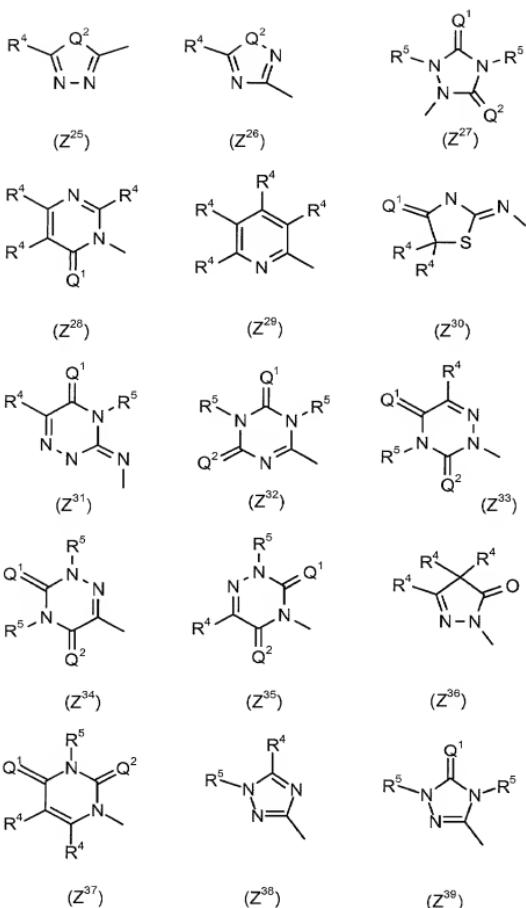


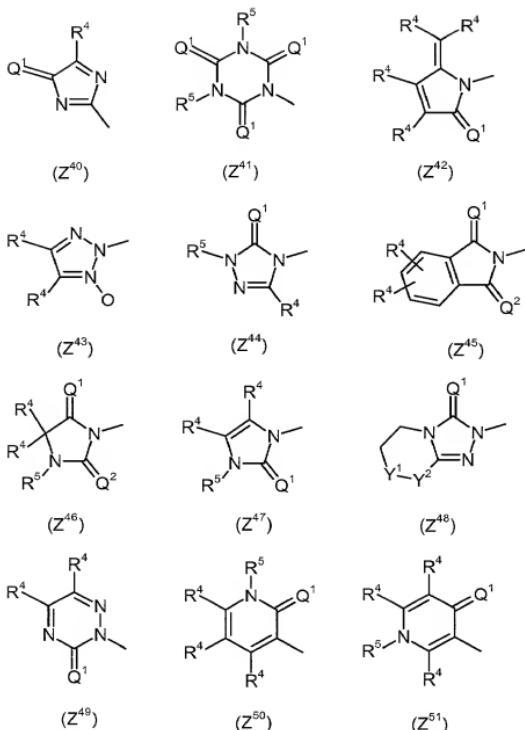
(Z⁵)



(Z⁶)







where

Q^1 represents O or S,

Q^2 represents O or S,

R^4 represents hydrogen, amino, nitro, cyano, carboxyl, carbamoyl, thiocarbamoyl, halogen, alkyl which has 1 to 6 carbon atoms and which is

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optionally substituted by cyano, halogen or C₁-C₄-alkoxy, or represents alkenyl or alkynyl, each of which has 2 to 6 carbon atoms and each of which is optionally substituted by halogen or represents alkoxy or alkoxycarbonyl, each of which has 1 to 6 carbon atoms in the alkyl groups and each of which is optionally substituted by cyano, halogen or C₁-C₄-alkoxy, or represents alkenyloxy or alkynyloxy, each of which has 3 to 6 carbon atoms and each of which is optionally substituted by halogen, or represents alkylthio which has 1 to 6 carbon atoms which is optionally substituted by cyano, halogen or C₁-C₄-alkoxy, or represents alkenylthio or alkynylthio, each of which has 3 to 6 carbon atoms and each of which is optionally substituted by halogen, or represents alkylamino or dialkylamino, each of which has 1 to 6 carbon atoms in the alkyl groups, or represents cycloalkyl or cycloalkylalkyl, each of which has 3 to 6 carbon atoms in the cycloalkyl groups and, if appropriate, 1 to 4 carbon atoms in the alkyl moiety and each of which is optionally substituted by cyano, halogen or C₁-C₄-alkyl,

R⁵ represents hydrogen, hydroxyl, amino, cyano, or represents alkyl, alkoxy, alkoxycarbonyl or alkylamino, each of which has up to 6 carbon atoms and each of which is optionally substituted by cyano, halogen or C₁-C₄-alkoxy, or represents alkenyl or alkynyl, each of which has up to 6 carbon atoms and each of which is optionally substituted by halogen, or represents cycloalkyl or cycloalkylalkyl, each of which has 3 to 6 carbon atoms in the cycloalkyl groups and, if appropriate 1 to 4 carbon atoms in the alkyl moiety and each of which is optionally substituted by cyano, halogen or C₁-C₄-alkyl, or represents phenyl or phenyl-C₁-C₄-alkyl each of which is optionally substituted by nitro, cyano, halogen, C₁-C₄-alkyl, C₁-C₄-halogenoalkyl, C₁-C₄-alkoxy or C₁-C₄-halogenoalkoxy,

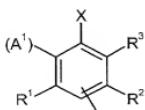
Y¹ represents O, S, SO, SO₂, NH, N(alkyl) or methylene and

Y^2 represents a single bond or O, S, SO, SO₂, NH or N(alkyl),

Y^1 and Y^2 being different in each individual case,

and

Z^2 represents the following group



where

R^1 is hydrogen, nitro, cyano or halogen,

R^2 is nitro, hydroxyl, cyano, carbamoyl, thiocarbamoyl, or represents alkyl or alkoxy, each of which has 1 to 4 carbon atoms and each of which is optionally substituted by halogen, and

R^3 represents nitro, hydroxyl, mercapto, amino, hydroxyamino, cyano, carboxyl, carbamoyl, thiocarbamoyl, halogen, or represents alkyl, alkylcarbonyl, alkoxy, alkoxy carbonyl, alkylthio, alkylsulphinyl, alkylsulphonyl or alkylamino, each of which has 1 to 6 carbon atoms in the alkyl groups and each of which is optionally substituted by cyano, carboxyl, carbamoyl, halogen, C₁-C₄-alkoxy or C₁-C₄-alkoxy-carbonyl, or represents alkylsulphonylamino, N,N-bis-alkylsulphonyl-amino, N-alkylcarbonyl-N-alkylsulphonyl-amino, each of which has 1 to 4 carbon atoms in the alkyl groups and each of which is optionally substituted by halogen, or represents alkenyl, alkenyloxy, alkenylthio, alkenylamino, alkinyl, alkinyloxy, alkinylthio, alkinylamino, each of which has up to 6

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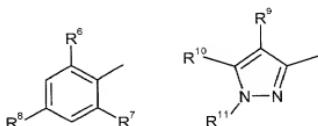
carbon atoms in the alkenyl or alkynyl groups and each of which is optionally substituted by cyano, carboxyl, carbamoyl, halogen or C₁-C₄-alkoxy-carbonyl, or represents cycloalkyl, cycloalkyloxy, cycloalkylthio, cycloalkylamino, cycloalkylsulphonylamino, cycloalkylalkyl, cycloalkylalkoxy, cycloalkylalkylthio or cycloalkylalkylamino, each of which has 3 to 6 carbon atoms in the cycloalkyl groups and, if appropriate, 1 to 4 carbon atoms in the alkyl moieties and each of which is optionally substituted by cyano, carboxyl, carbamoyl, halogen or C₁-C₄-alkyl, or represents aryl, aryloxy, arylthio, arylamino, arylalkyl, arylalkoxy, arylalkylthio, arylalkylamino, N-arylcarbonyl-N-alkylsulphonyl-amino, each of which has 6 or 10 carbon atoms in the aryl groups and, if appropriate, 1 to 4 carbon atoms in the alkyl moiety and each of which is optionally substituted by nitro, cyano, carboxyl, carbamoyl, halogen, C₁-C₄-alkyl, C₁-C₄-halogenoalkyl, C₁-C₄-alkoxy, C₁-C₄-halogenoalkoxy or C₁-C₄-alkoxy-carbonyl, and

X represents hydrogen or halogen.

21. (Amended) A s[S]ubstance according to Claim 19, [characterized in that]
wherein

A¹ represents O,

Z¹ represents one of the following groups



where

T U S E C O = 3 3 2 4 6 8 6 0

R⁶ represents cyano or halogen,

R⁷ represents hydrogen or halogen,

R⁸ represents cyano, halogen, or represents halogenoalkyl or halogenoalkoxy, each of which has 1 to 4 carbon atoms,

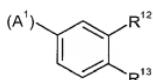
R⁹ represents hydrogen or halogen,

R¹⁰ represents halogenoalkyl or halogenoalkoxy, each of which has 1 to 4 carbon atoms, and

R¹¹ represents alkyl having 1 to 4 carbon atoms,

and

Z² represents the following group

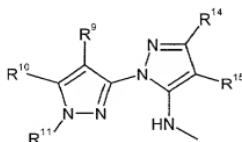


wherein [in which]

R¹² represents carboxyl, or represents alkoxy or alkoxy carbonyl, each of which has up to 4 carbon atoms and each of which is optionally substituted by cyano, halogen or C₁-C₄-alkoxy, and

R¹³ represents cyano, carbamoyl, thiocarbamoyl, halogen or represents halogenoalkyl or halogenoalkoxy, each of which has 1 to 4 carbon atoms.

22. (Amended) A s[S]ubstance according to Claim 19, [characterized in that]
wherein the substance has the following general formula:



wherein [in which]

[R⁹, R¹⁰ and R¹¹ are as defined in Claim 20]

R⁹ represents hydrogen or halogen,

R¹⁰ represents halogenoalkyl or halogenoalkoxy, each of which has 1 to 4 carbon atoms, and

R¹¹ represents alkyl having 1 to 4 carbon atoms,

R¹⁴ represents hydrogen, halogen or alkyl having 1 to 4 carbon atoms, and

R¹⁵ represents nitro, cyano, carbamoyl, thiocarbamoyl, or represents alkyl or alkoxy, each of which has 1 to 4 carbon atoms and each of which is optionally substituted by cyano, halogen or C₁-C₄-alkoxy.

23. (Amended) An a[A]ssay system comprising

- containers with mixtures as defined in Claim 1 [one of Claims 1 to 10],
- a device for irradiating the mixtures of plane-polarized light of a wavelength which excites the fluorescent substance to emit light, and
- a device for measuring the fluorescence polarization values or the anisotropy values of the light emitted.

25. (Amended) A s[S]ubstance[s] which interact with the enzyme protoporphyrinogen oxidase and which have been identified by the method according to Claim 1 [one of Claims 1 to 10].

Please add the following claims:

--26. A method according to Claim 2, wherein the PPO is a plant PPO.

27. A method according to Claim 2, wherein a PPO-containing plant cell extract is employed.

28. A method according to Claim 2, wherein biochemically purified PPO from plant cell extracts is employed.

29. A method according to Claim 2, wherein recombinantly produced PPO is employed.

30. A method according to Claim 2, wherein the fluorescent substance is a substance labelled with a fluorescent dye.

31. A method according to Claim 2, wherein the fluorescent substance has the following structure:



wherein "linker" represents a hydrocarbon chain which is in each case straight-chain or branched, in each case saturated or unsaturated, in each case optionally substituted, in each case linked at one end to the substance and at the other end to the fluorescent dye, it being possible for this hydrocarbon chain to contain in each case at the beginning or at the end or within the chain one or more of the following hetero components:

Q^1 , CQ^2 , CQ^2-Q^1 , Q^1-CQ^2 , $Q^1-CQ^2-Q^1$, SO , SO_2

wherein Q^1 and Q^2 represent O, S or NH, or represents a carbocyclic or heterocyclic group which is in each case saturated or unsaturated, in each case optionally substituted and linked at one end to the substance and at the other end to the fluorescent dye, and

"substance" represents a substance which is capable of interacting with PPO.

32. A substance which interact with the enzyme protoporphyrinogen oxidase and which have been identified by the method according to Claim 2.
33. A substance according to Claim 12, wherein
 - A represents O.
 - Q represents O.
 - X^1 represents hydrogen, fluorine, chlorine, bromine or alkyl having 1 to 4 carbon atoms, and
 - X^2 represents hydrogen, fluorine, chlorine, bromine or alkyl having 1 to 4 carbon atoms.
34. A substance according to Claim 11, wherein the carbocyclic or heterocyclic group of the linker has up to 10 carbon atoms and 1 or 2 oxygen or sulphur atoms.
35. A substance according to Claim 11, wherein the carbocyclic or heterocyclic group of the linker has up to 10 carbon atoms, up to 5 nitrogen atoms, and 1 or 2 oxygen or sulphur atoms.
36. An assay system comprising
 - a) containers with mixtures as defined in Claim 2,
 - b) a device for irradiating the mixtures of plane-polarized light of a wavelength which excites the fluorescent substance to emit light, and

- c) a device for measuring the fluorescence polarization values or the anisotropy values of the light emitted.--

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